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Nanoscale Changes in the Organisation of Junctional Proteins in JPH2 Transgenic Mice

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In cardiomyocytes, transverse tubule (t-tubule) and sarcoplasmic reticulum (SR) membranes form close associations, known as diadic junctions, which are critical for excitation-contraction (E-C) coupling. Ryanodine receptors (RyRs), which are the SR calcium release channels, are concentrated in junctions where they play a key role for calcium release. In human and animal models of heart failure junctions and transverse (t-) tubules often become disorganised but the underlying mechanisms remain unclear. The SR membrane protein junctophilin 2 (JPH2) has been implicated in junction formation and maintenance. In this study, transgenic mouse models were used to investigate the influence JPH2 expression on RyR organisation. Cardiomyocytes from adult mice in which JPH2 had been acutely knocked down or over-expressed and control cells labeled for RyR and JPH2. Confocal laser scanning and optical super-resolution microscopy were used to investigate junctional protein organisation and t-tubule architecture.

As previously observed in response to JPH2 knockdown t-tubule organisation was disrupted. Regular t-tubules in line with the z-discs were predominant in the control cardiomyocytes, but greatly reduced and irregular in JPH2 knockdown cells. In JPH2 overexpressing myocytes the t-tubules and longitudinal tubules were more abundant.

RyR organisation was also altered in response to changes in JPH2 expression. Large RyR superclusters (>200nm diameter) were present in control cells and more abundant in JPH2 over-expressing cardiomyocytes. Such superclusters were mostly absent from JPH2 knockdown cells in which smaller clusters were observed. These changes only became apparent with the resolution provided by optical super-resolution but were difficult to detect with conventional confocal imaging. Our results indicate that cardiomyocyte organisation is severely affected by altered expression of JPH2 and raise questions on the functional role of RyR superclusters and their effect on local and global Ca^{2+} release.

Platform: Other Channels

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A Dimeric Dual-Topology Microbial Fluoride Channel

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Fluoride ion has pervaded the environment (10-100 micromolar in sea, ground water, and soil) over evolutionary time, and inhibits several essential phosphoryl group transfer enzymes involved in energy metabolism and nucleic acid synthesis with K_i values around 100 micromolar. Widespread microorganisms have evolved defenses against F^- ion, including a newly discovered family of membrane transport proteins composed of four transmembrane helices, called Flucs. Using single channel electrical recordings of purified, reconstituted proteins in planar lipid bilayers, we show that Flucs are constitutively open electrodiffusive F^- channels, with main state conductance of ~10 pS, and possess unprecedentedly high and biologically required 10,000-fold selectivity for F^- over Cl^-. Using SEC-UV/RI/LS, single molecule TIRF photobleaching, and a "Poisson dump" assay for calculating the molecular weight of membrane transporters in liposomes, we show that Flucs function as homodimers, an unusually small number of subunits for a channel. For deeper mechanistic studies, we have selected a panel of "monobody" binders to Fluc proteins from a phage-display library of 10^{10} fibronection-domain scaffolds with randomly varied loops. These monobodies block single-Fluc F^- current with nanomolar affinity and simple bimolecular kinetics, and most likely inhibit F^- current by blocking the pore. In single channel recordings, we show that the monobodies block the same single Fluc channel from both sides of the bilayer, which can only occur if the subunits of the dimer are arranged in an antiparallel orientation relative to each other, and thus present the same epitope on both sides of the membrane. This result emphatically supports earlier indications of antiparallel topology in Flucs, including lysine cross-linking experiments and genetically fused constructs. Dual-topology architecture is a surprising departure from the familiar barrel-stave plan that many other channels are built on, but is reminiscent of inverted structural repeats in modern-day transporters.

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A Repulsion Mechanism Explains Magnesium Permeation and Selectivity in CorA

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Magnesium (Mg^{2+}) plays a central role in biology, regulating the activity of many enzymes and stabilizing the structure of key macromolecules. In prokaryotes, the homopentameric membrane protein CorA is the primary source of Mg^{2+} uptake and its net influx is self-regulated by the intracellular Mg^{2+} concentration. Using electrophysiological methods applied on a Mg^{2+}-dependent gating mutant, we were able to characterize CorA selectivity and permeation properties to both monovalent and divalent cations under perfused Two Electrode Voltage Clamp. We show that under physiological conditions, CorA is a multiply-occupied Mg^{2+} channel, able to fully exclude monovalent cations, and displaying mild selectivity against other divalents according to the sequence $Ca^{2+} > Mn^{2+} > Co^{2+} > Mg^{2+} > Ni^{2+}$. Cobalt hexammine, a structural analog of hydrated Mg^{2+}, blocks Mg^{2+} currents with a shallow voltage dependence, placing the binding site at the extracellular mouth of the pore. Selectivity against monovalent cations takes place via divalent ion binding at a high affinity site formed by the GMN signature sequence (G312 and N314) at the extracellular side of the pore. Molecular dynamics simulations tend to support this conclusion while reconciling the pentameric geometry of CorA with the expected hexacoordination of Mg^{2+}. The mechanism presented here is reminiscent of repulsion models proposed for Ca^{2+} channel selectivity in spite of differences in sequence and overall structure.

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Mechanisms of Molecular Transport through the Proton-Gated Urea Channel of Helicobacter Pylori

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Helicobacter pylori survival in acidic environments relies on cytoplasmic hydrolysis of gastric urea into ammonia and carbon dioxide, which buffer the pathogen's periplasm. Urea uptake is greatly enhanced and regulated by HpUreI, a proton-gated inner-membrane channel protein essential for gastric survival of H. pylori. The crystal structure of HpUreI describes a static snapshot of the channel with two constriction sites near the center of the bilayer that are too narrow to allow passage of urea or even water. Here we report the atomic resolution urea transport mechanism, revealed by unrestrained microsecond equilibrium molecular dynamics simulations of the hexameric channel assembly. Two consecutive constrictions open to allow conduction of urea, which is guided through the channel by an interplay of conserved residues that determine proton rejection and solute selectivity. Remarkably, HpUreI conducts water at rates equivalent to aquaporins, which might be essential for efficient transport of urea at small concentration gradients.

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Modulation of the Bacterial Mechanosensitive Channel of Small Conductance (MSCS) by Cardiolipin - Electrophysiological, Alanine Mutagenesis and Modelling Studies

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The bacterial mechanosensitive channel of Small (MscS) conductance responds to membrane tension by opening when the bacterium experiences hypo-osmotic shock conditions to prevent cell lysis [1]. Environmental factors such as cholesterol [2] and cations/anions [3] also affect the gating behaviour of this channel. Here we report an alanine mutagenesis study using both a computational and experimental approach to investigate the interaction of charged protein residues with lipid headgroups. The computational results on the open structure of MscS show that residues K60 and R46 in TM1 helix interact strongly with cardiolipin (CDL, Fig 1A), while in the closed conformation only R46 interacts with cardiolipin (Fig 1B). Results from Patch Clamp recordings on